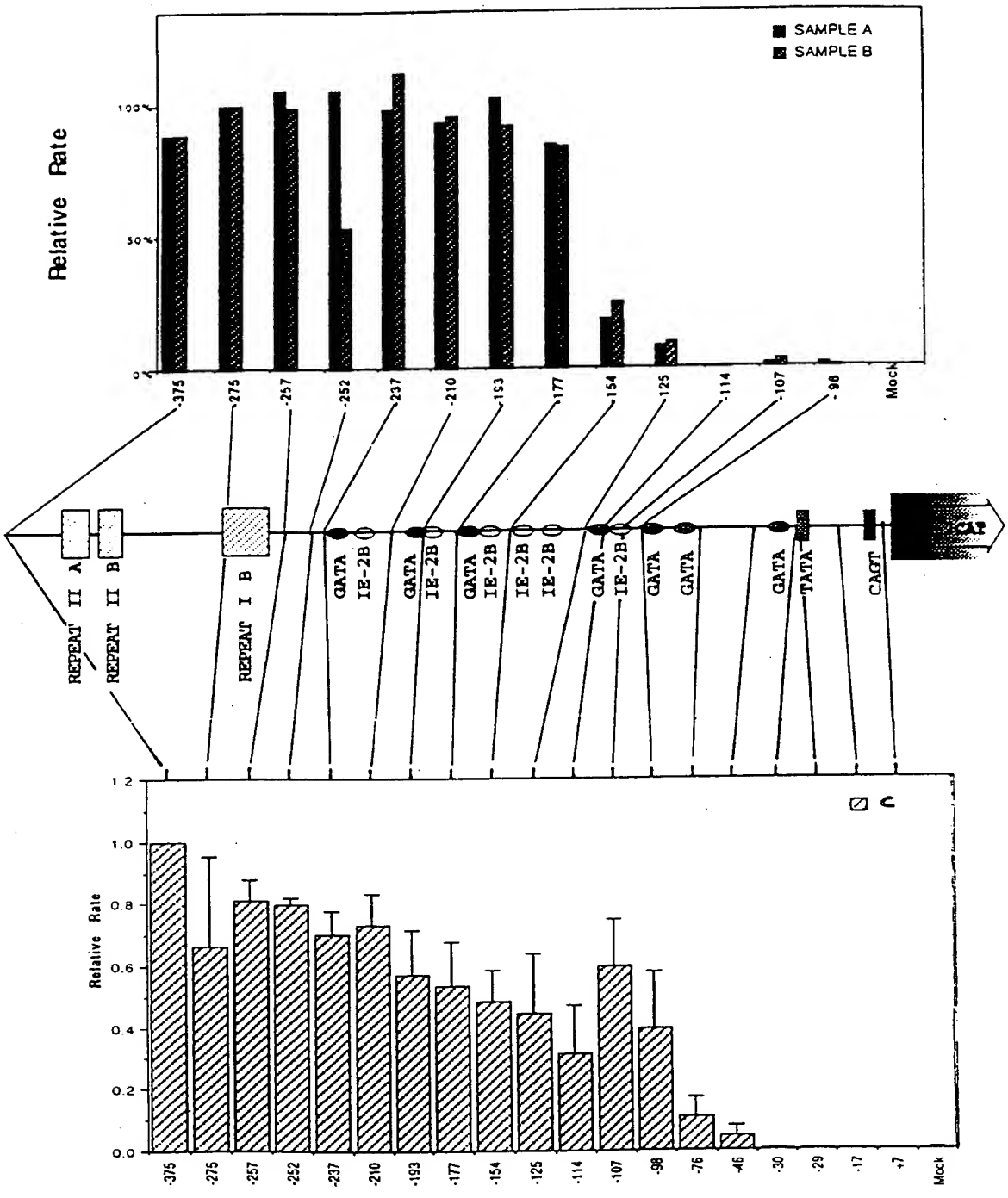
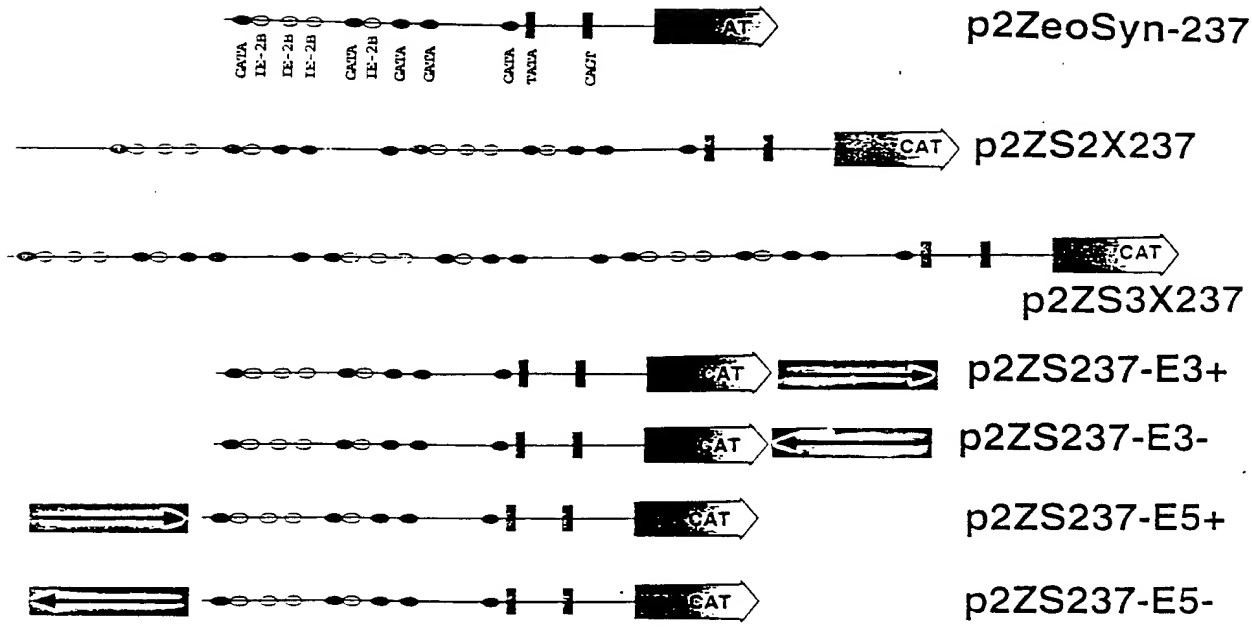

SAMPLE A
SAMPLE B
Relative Rate
100
50
0
-375
-275
-257
-252
-237
-210
-193
-177
-154
-125
-114
-107
-98
Mock
REPEAT II A
REPEAT II B
REPEAT I B
GATA
IE-2B
TATA
CAAT
CAT
C
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Relative Rate
-76
-46
-30
-29
-17
-7

Figure 1a


p2ZeoSyn-237
p2ZS2X237
p2ZS3X237
p2ZS237-E3+
p2ZS237-E3-
p2ZS237-E5+
p2ZS237-E5-
CAT

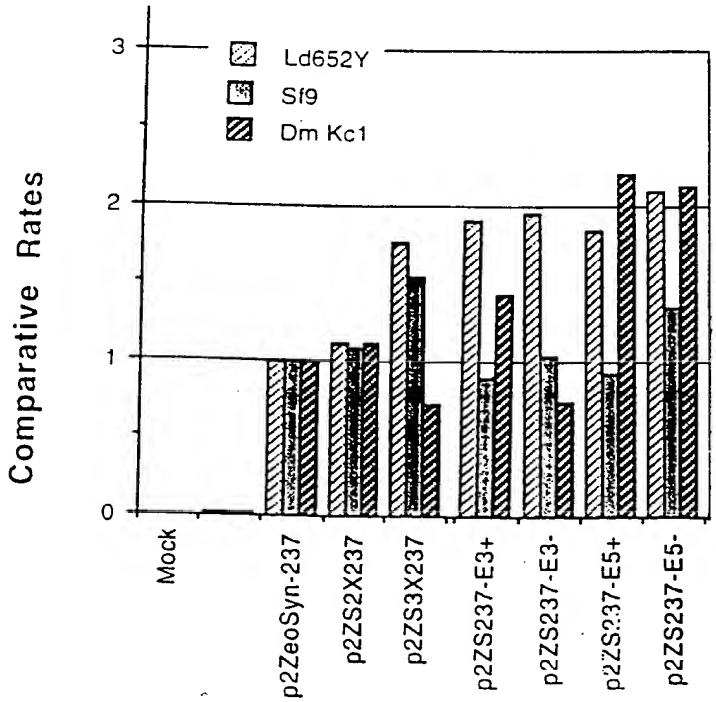

Comparative Rates
Ld652Y
Sf9
Dm Kc1
0
1
2
3
Mock
p2ZeoSyn-237
p2ZS2X237
p2ZS3X237
p2ZS237-E3+
p2ZS237-E3-
p2ZS237-E5+
p2ZS237-E5-

Figure 1b

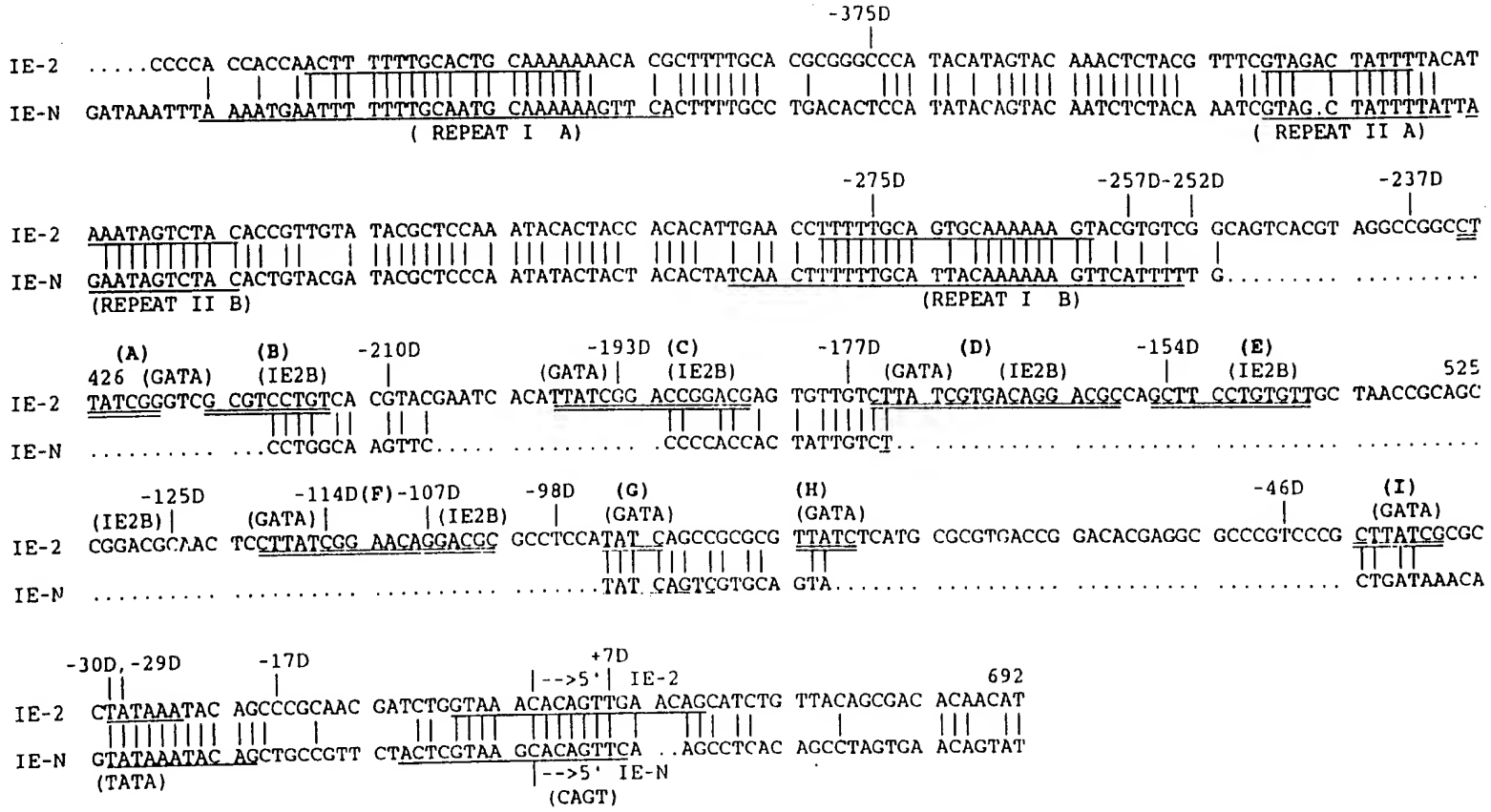

-375D
IE-2 .....CCCCA CCACCAACTT TTTTGCACTG CAAAAAAACA CGCTTTTGCA CGCGGGCCCA TACATAGTAC AAACTCTACG TTTCGTAGAC TATTTTACAT
IE-N GATAAATTTA AAATGAATTT TTTTGCAATG CAAAAAAGTT CACTTTTGCC TGACACTCCA TATACAGTAC AATCTCTACA AATCGTAG.C TATTTTATTA
(REPEAT I A)
(REPEAT II A)
-275D
-257D-252D
-237D
IE-2 AAATAGTCTA CACCGTTGTA TACGCTCCAA ATACACTACC ACACATTGAA CCTTTTTTGCA GTGCAAAAAA GTACGTGTCG GCAGTCACGT AGGCCGGCCT
IE-N GAATAGTCTA CACTGTACGA TACGCTCCCA ATATACTACT ACACTATCAA CTTTTTTGCA TTACAAAAAA GTTCATTTTT G..... .....
(REPEAT II B)
(REPEAT I B)
(A)
(B)
-210D
-193D (C)
-177D
(D)
-154D
(E)
426 (GATA)
(IE2B)
(GATA)
(IE2B)
(GATA)
(IE2B)
(IE2B)
525
IE-2 TATCGGGTCG CGTCCTGTCA CGTACGAATC ACATTATCGG ACCGGACGAG TGTTGTCTTA TCGTGACAGG ACGCCAGCTT CCTGTGTTGC TAACCGCAGC
IE-N ....CCTGGCA AGTTC..... ..... .CCCCACCAC TATTGTCT.. ....
-125D
-114D(F)-107D
-98D
(G)
(H)
-46D
(I)
(IE2B)
(GATA)
(IE2B)
(GATA)
(GATA)
(GATA)
IE-2 CGGACGCAAC TCCTTATCGG AACAGGACGC GCCTCCATAT CAGCCGCGCG TTATCTCATG CGCGTGACCG GACACGAGGC GCCCGTCCCG CTTATCGCGC
IE-N ....TAT CAGTCGTGCA GTA..... .... CTGATAAACA
-30D,-29D
-17D
+7D
-->5'
IE-2
692
IE-2 CTATAAATAC AGCCCGCAAC GATCTGGTAA ACACAGTTGA ACAGCATCTG TTACAGCGAC ACAACAT
IE-N GTATAAATAC AGCTGCCGTT CTACTCGTAA GCACAGTTCA ..AGCCTCAC AGCCTAGTGA ACAGTAT
(TATA)
-->5' IE-N
(CAGT)

Figure 2a

TAGGGG GGGGGGG

A	-232	C	T	T	A	T	C	G	G										-225
B	-212R									G	A	C	A	G	G	A	C	G	C	-221R
C	-197		T	T	A	T	C	G	G	.	A	C	[C]	G	G	A	C	G		-191
D	-175	C	T	T	A	T	C	G	[T]	G	A	C	A	G	G	A	C	G	C	-162
E	-147R									A	A	C	A	G	G	A	[A]	G	C	-157R
F	-118	C	T	T	A	T	C	G	G	A	A	C	A	G	G	A	C	G	C	-101
G	-93			T	A	T	C													-89
H	-80		T	T	A	T	C													-76
I	-40	C	T	T	A	T	C	G												-34
Consensus		C	T	T	A	T	C	G	G	-	A	C	A	G	G	A	C	G	C	

Figure 2b

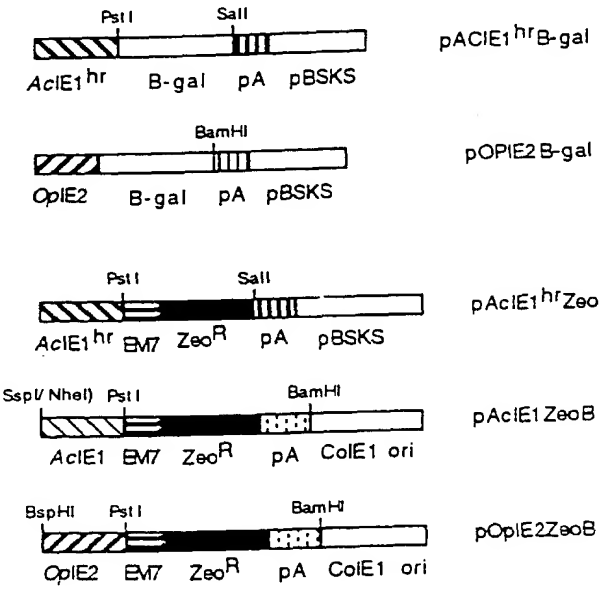

PstI
SalI
AclE1hr
B-gal
pA
pBSKS
pAClE1hrB-gal
BamHI
OplE2
B-gal
pA
pBSKS
pOPlE2 B-gal
PstI
SalI
AclE1hr
EM7
Zeo^R
pA
pBSKS
pAclE1hrZeo
(SspI/NheI)
PstI
BamHI
AclE1
EM7
Zeo^R
pA
ColE1 ori
pAclE1 ZeoB
BspHI
PstI
BamHI
OplE2
EM7
Zeo^R
pA
ColE1 ori
pOplE2ZeoB

Figure 3

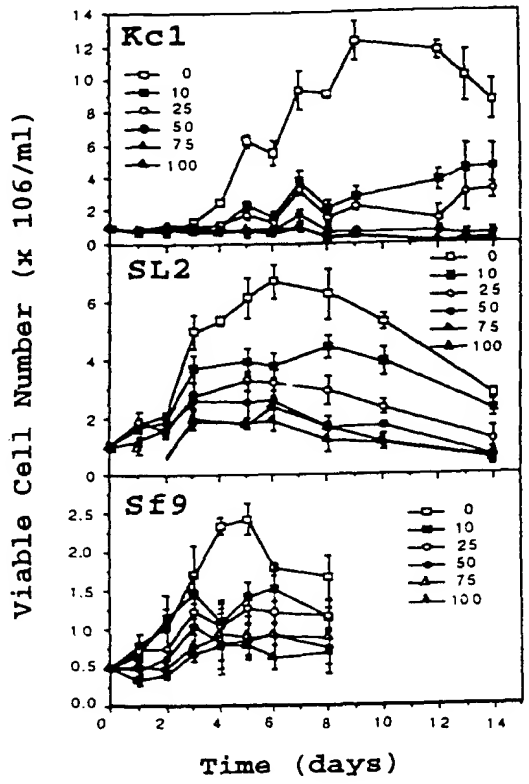

Kc1
0
10
25
50
75
100
SL2
0
10
25
50
75
100
Sf9
0
10
25
50
75
100
Viable Cell Number (x 106/ml)
Time (days)

Figure 4a

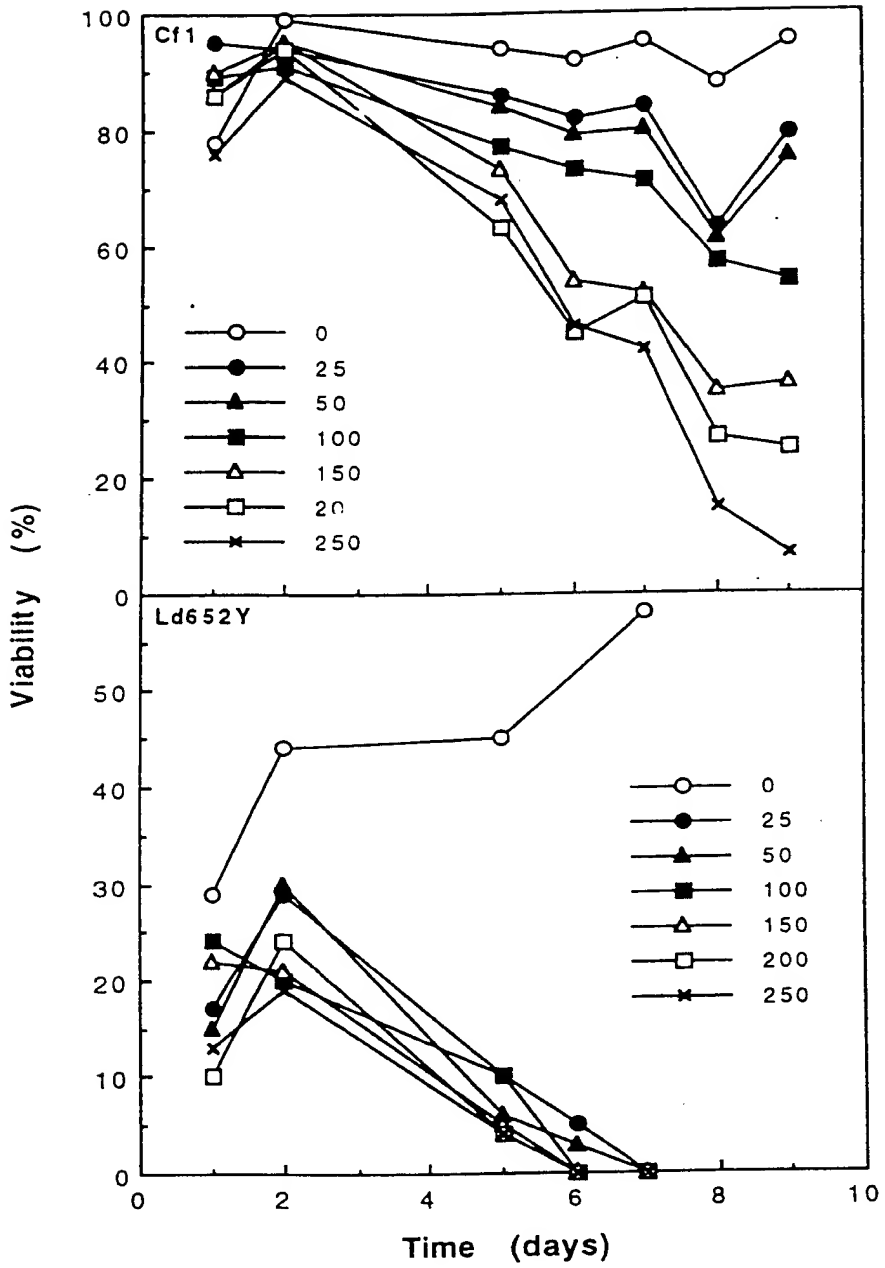


Figure 4b

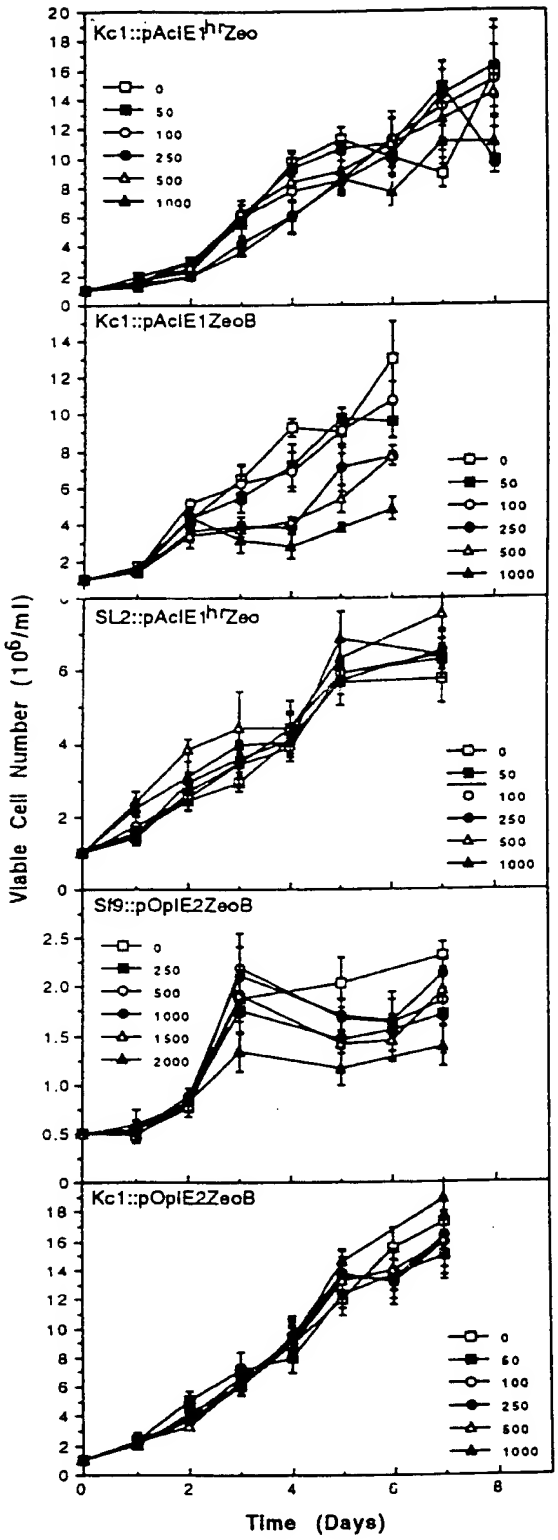

Kc1::pAclE1hrZeo
0
50
100
250
500
1000
Kc1::pAclE1ZeoB
0
50
100
250
500
1000
SL2::pAclE1hrZeo
0
50
100
250
500
1000
Sf9::pOplE2ZeoB
0
250
500
1000
1500
2000
Kc1::pOplE2ZeoB
0
50
100
250
500
1000
Viable Cell Number (10^6/ml)
Time (Days)

Figure 5

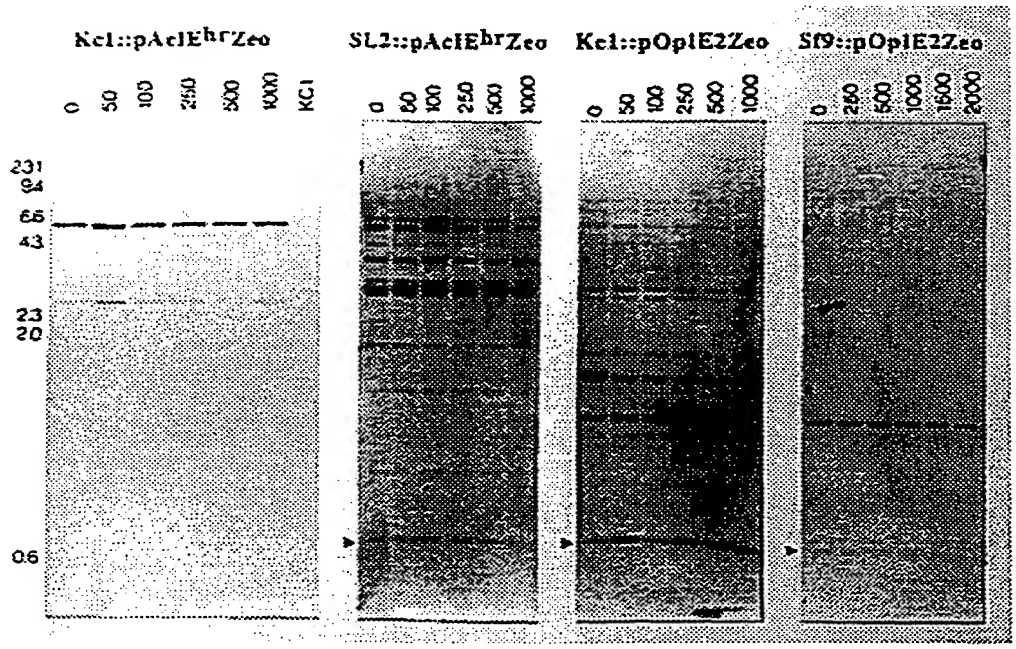

Kc1::pAclE^hrZeo
SL2::pAclE^hrZeo
Kc1::pOplE2Zeo
Sf9::pOplE2Zeo
0
50
100
250
500
1000
KC1
0
50
100
250
500
1000
0
50
100
250
500
1000
0
250
500
1000
1500
2000
231
94
66
43
23
20
0.6

Figure 6

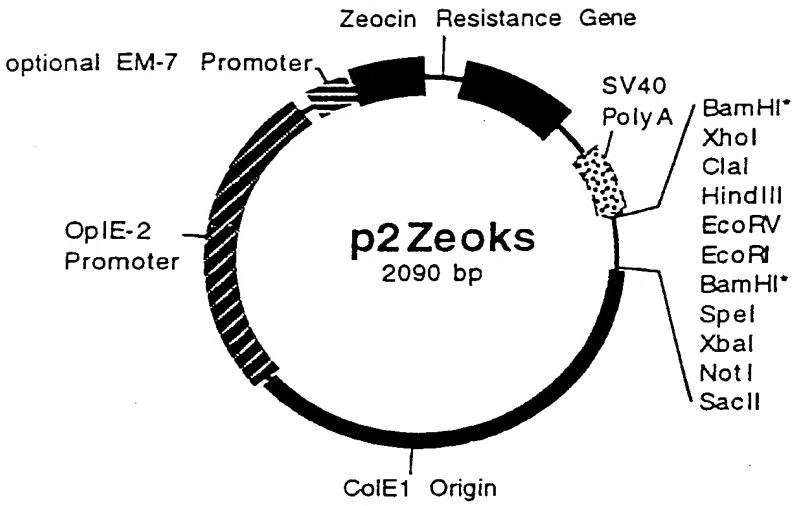

Zeocin Resistance Gene
optional EM-7 Promoter
SV40
PolyA
BamHI*
XhoI
ClaI
HindIII
EcoRV
EcoRI
BamHI*
SpeI
XbaI
NotI
SacII
OpIE-2
Promoter
p2Zeoks
2090 bp
ColE1 Origin

Figure 7

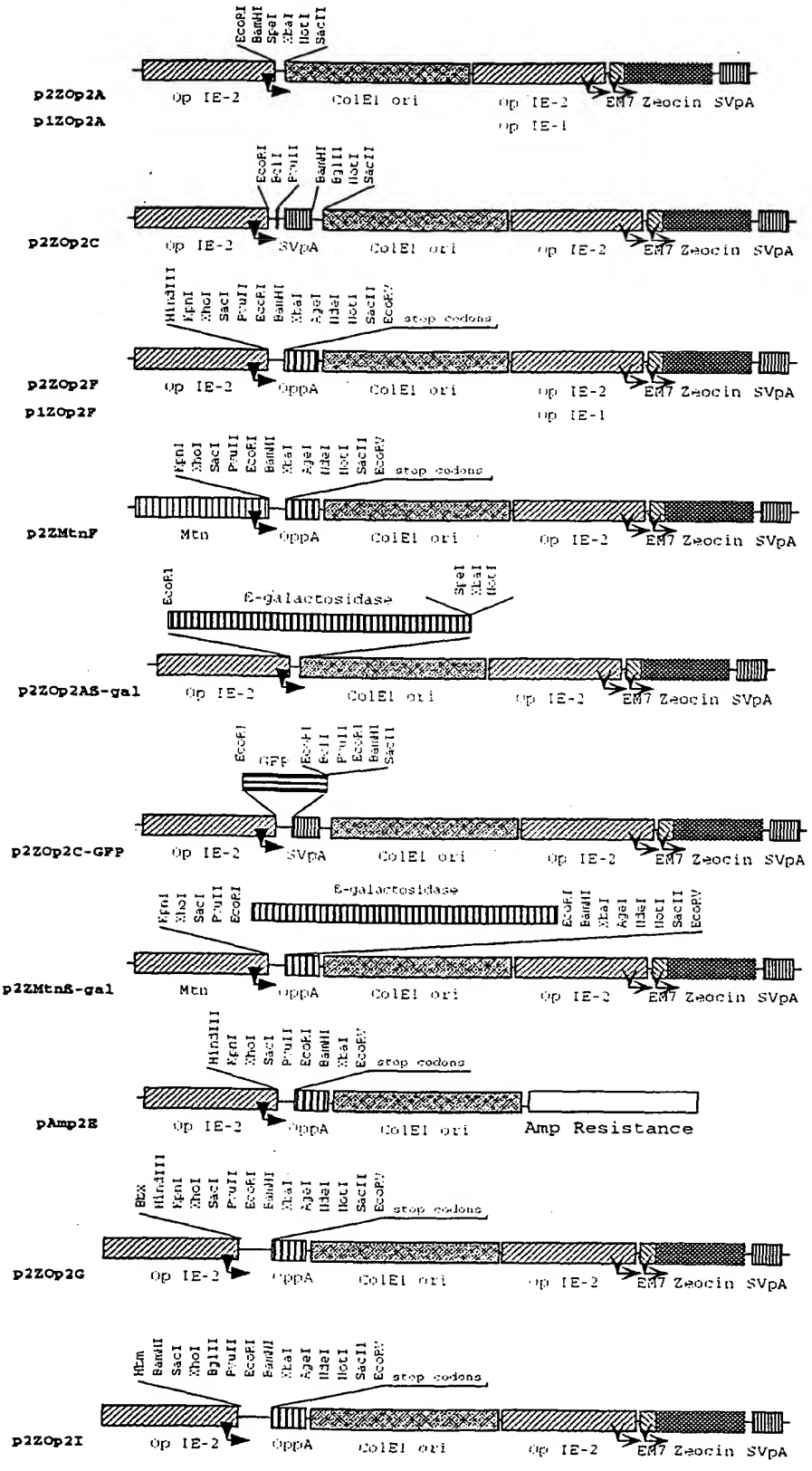

p2ZOp2A
p1ZOp2A
Op IE-2
ColE1 ori
Op IE-2
Op IE-1
EM7 Zeocin SVpA
p2ZOp2C
SVpA
p2ZOp2F
p1ZOp2F
OppA
stop codons
p2ZMtnF
Mtn
p2ZOp2AS-gal
β-galactosidase
p2ZOp2C-GFP
GFP
p2ZMtnS-gal
pAmp2E
Amp Resistance
p2ZOp2G
p2ZOp2I

Figure 8a

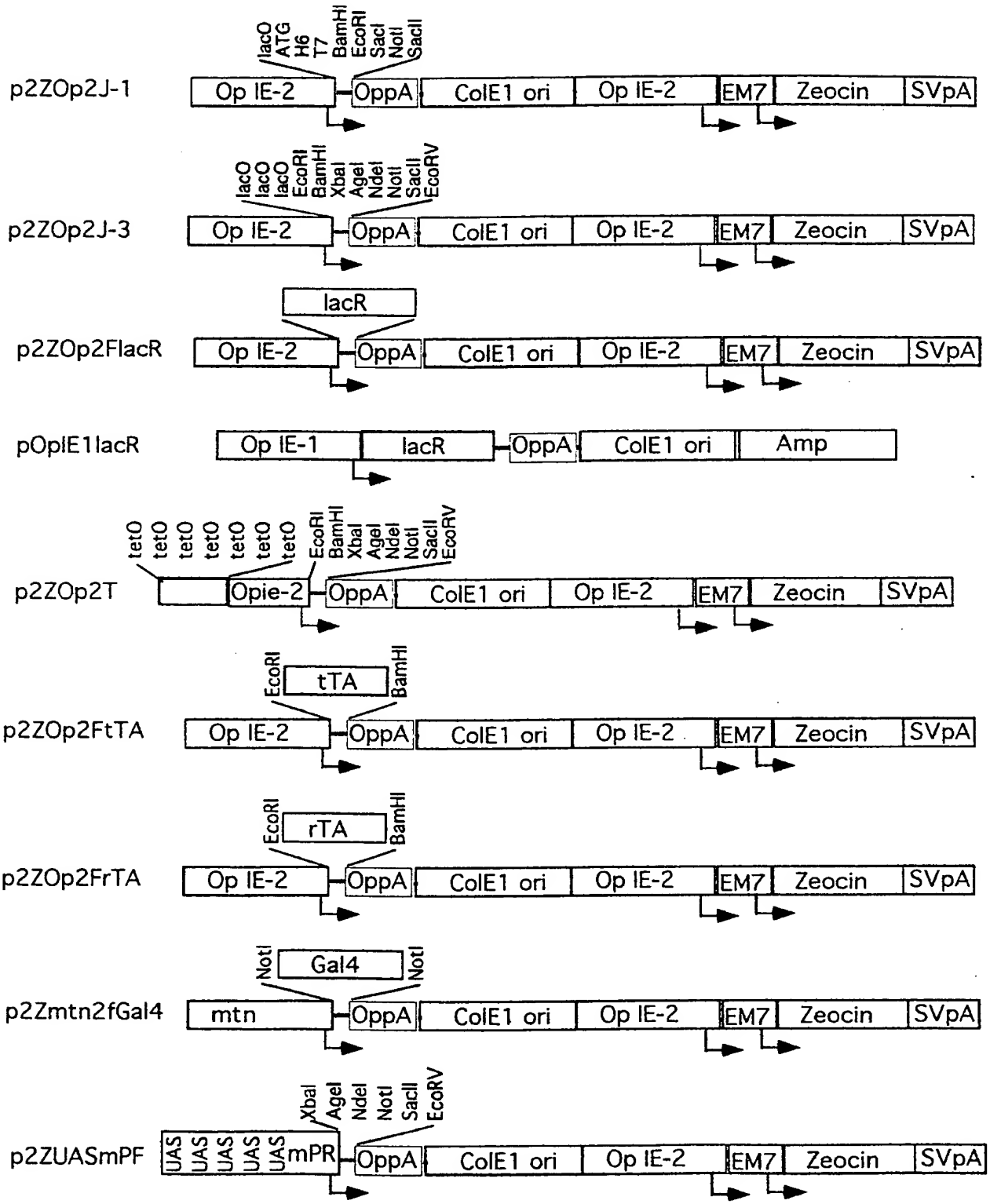
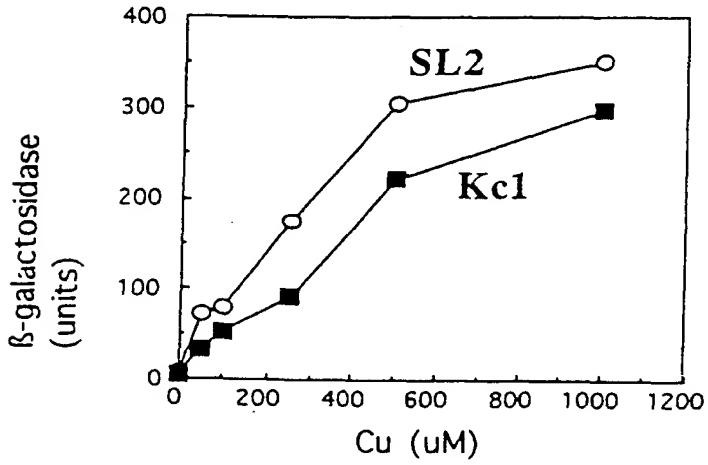

lacO
ATG
H6
T7
BamHI
EcoRI
SacI
NotI
SacII
p2ZOp2J-1
Op IE-2
OppA
ColE1 ori
Op IE-2
EM7
Zeocin
SVpA
lacO
lacO
lacO
EcoRI
BamHI
XbaI
AgeI
NdeI
NotI
SacII
EcoRV
p2ZOp2J-3
Op IE-2
OppA
ColE1 ori
Op IE-2
EM7
Zeocin
SVpA
lacR
p2ZOp2FlacR
Op IE-2
OppA
ColE1 ori
Op IE-2
EM7
Zeocin
SVpA
pOpIE1lacR
Op IE-1
lacR
OppA
ColE1 ori
Amp
tetO
tetO
tetO
tetO
tetO
tetO
tetO
EcoRI
BamHI
XbaI
AgeI
NdeI
NotI
SacII
EcoRV
p2ZOp2T
Opie-2
OppA
ColE1 ori
Op IE-2
EM7
Zeocin
SVpA
EcoRI
tTA
BamHI
p2ZOp2FtTA
Op IE-2
OppA
ColE1 ori
Op IE-2
EM7
Zeocin
SVpA
EcoRI
rTA
BamHI
p2ZOp2FrTA
Op IE-2
OppA
ColE1 ori
Op IE-2
EM7
Zeocin
SVpA
NotI
Gal4
NotI
p2Zmtn2fGal4
mtn
OppA
ColE1 ori
Op IE-2
EM7
Zeocin
SVpA
XbaI
AgeI
NdeI
NotI
SacII
EcoRV
p2ZUASmPF
UAS
UAS
UAS
UAS
UAS
mPR
OppA
ColE1 ori
Op IE-2
EM7
Zeocin
SVpA

Figure 8b


400
300
200
100
0
ß-galactosidase
(units)
SL2
Kc1
0
200
400
600
800
1000
1200
Cu (uM)

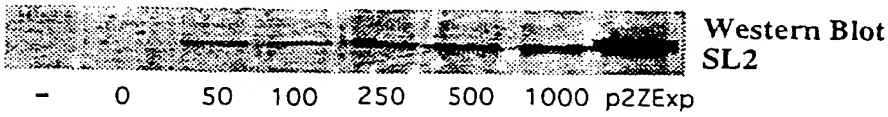

Western Blot
SL2
-
0
50
100
250
500
1000
p2ZExp

Figure 9

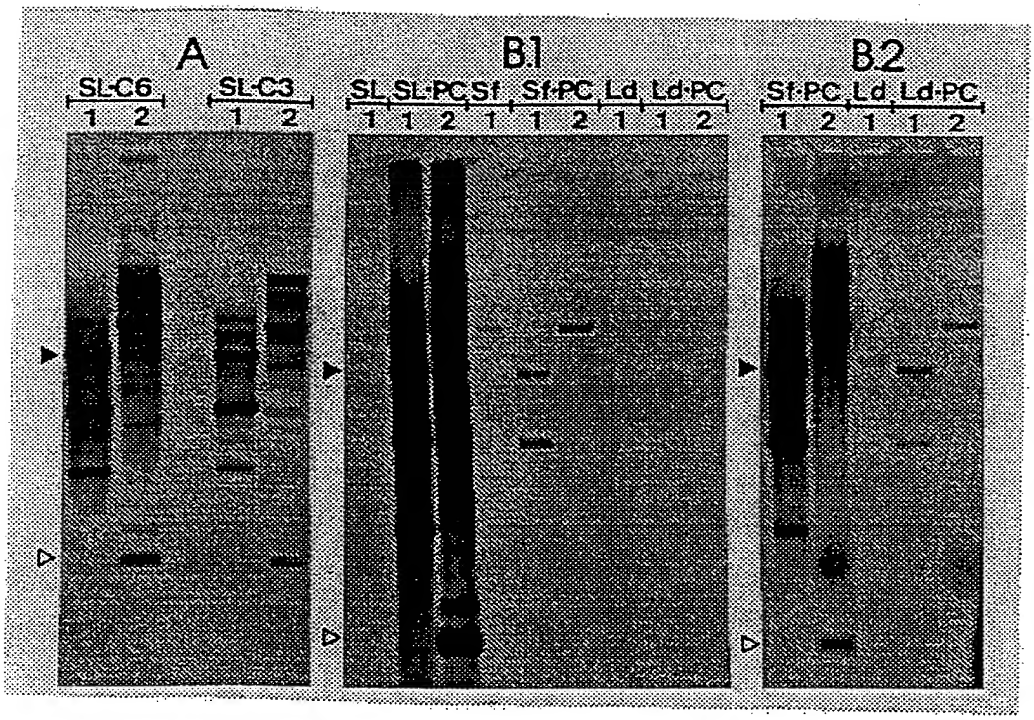

A
SL-C6
1 2
SL-C3
1 2
B1
SL
1
SL-PC
1 2
Sf
1
Sf-PC
1 2
Ld
1
Ld-PC
1 2
B2
Sf-PC
1 2
Ld
1
Ld-PC
1 2

Figure 10

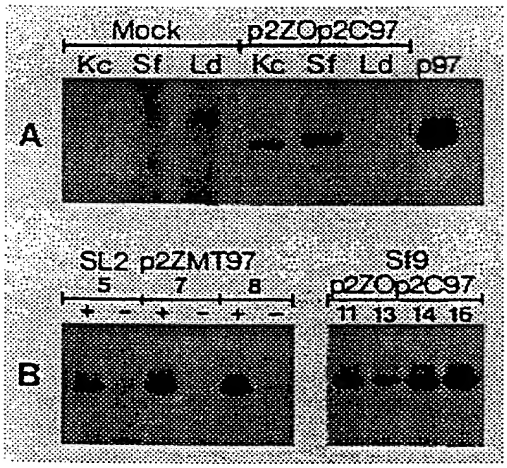

Mock
p2ZOp2C97
Kc Sf Ld Kc Sf Ld p97
A
SL2 p2ZMT97
5 7 8
+ − + − + −
Sf9
p2ZOp2C97
11 13 14 16
B

Figure 11

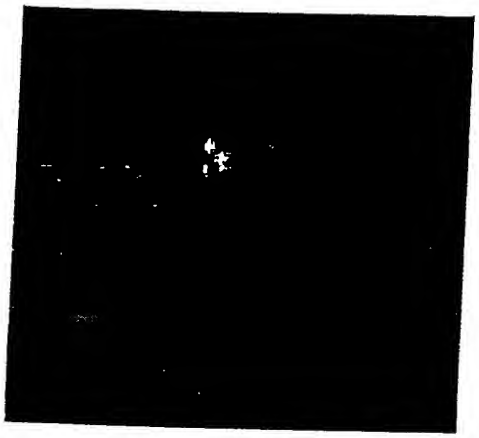


Figure 12a

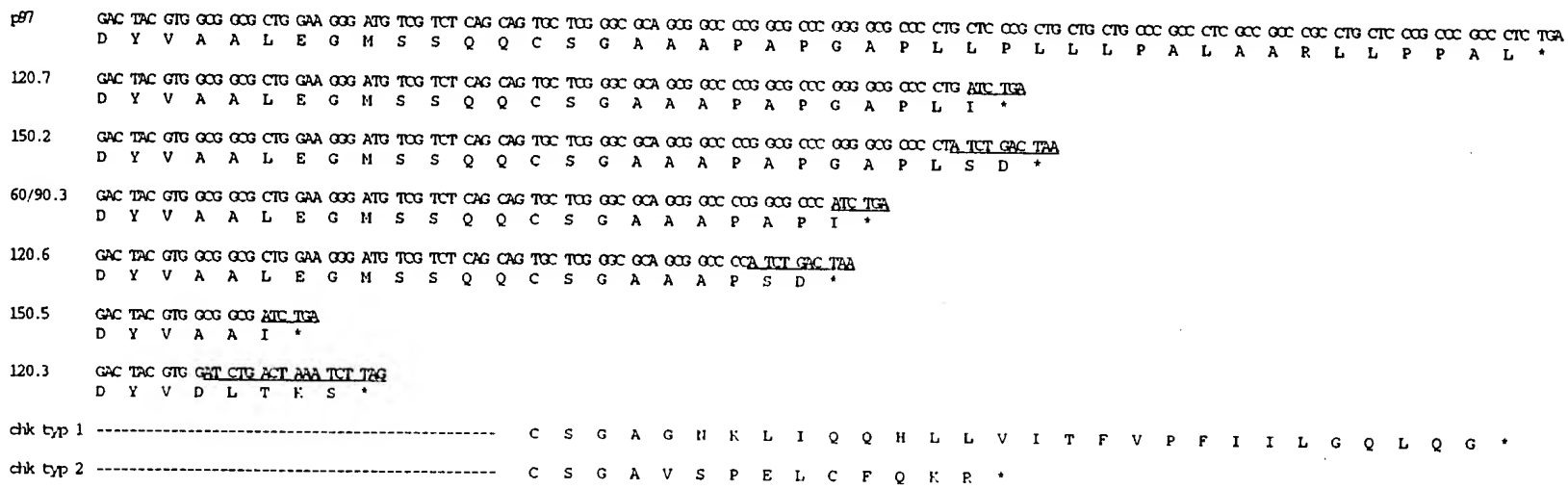

p97
GAC TAC GTG GCG GCG CTG GAA GGG ATG TCG TCT CAG CAG TGC TCG GGC GCA GCG GCC CCG GCG CCC GGG GCG CCC CTG CTC CCG CTG CTG CTG CCC GCC CTC GCC GGC CGC CTG CTC CGG CCC GCC CTC TGA
D Y V A A L E G M S S Q Q C S G A A A P A P G A P L L P L L L P A L A A R L L P P A L *
120.7
GAC TAC GTG GCG GCG CTG GAA GGG ATG TCG TCT CAG CAG TGC TCG GGC GCA GCG GCC CCG GCG CCC GGG GCG CCC CTG ATC TGA
D Y V A A L E G M S S Q Q C S G A A A P A P G A P L I *
150.2
GAC TAC GTG GCG GCG CTG GAA GGG ATG TCG TCT CAG CAG TGC TCG GGC GCA GCG GCC CCG GCG CCC GGG GCG CCC CTA TCT GAC TAA
D Y V A A L E G M S S Q Q C S G A A A P A P G A P L S D *
60/90.3
GAC TAC GTG GCG GCG CTG GAA GGG ATG TCG TCT CAG CAG TGC TCG GGC GCA GCG GCC CCG GCG CCC ATC TGA
D Y V A A L E G M S S Q Q C S G A A A P A P I *
120.6
GAC TAC GTG GCG GCG CTG GAA GGG ATG TCG TCT CAG CAG TGC TCG GGC GCA GCG GCC CCA TCT GAC TAA
D Y V A A L E G M S S Q Q C S G A A A P S D *
150.5
GAC TAC GTG GCG GCG ATC TGA
D Y V A A I *
120.3
GAC TAC GTG GAT CTG ACT AAA TCT TAG
D Y V D L T K S *
chk typ 1 ---------- C S G A G N K L I Q Q H L L V I T F V P F I I L G Q L Q G *
chk typ 2 ---------- C S G A V S P E L C F Q K R *

Figure 12b

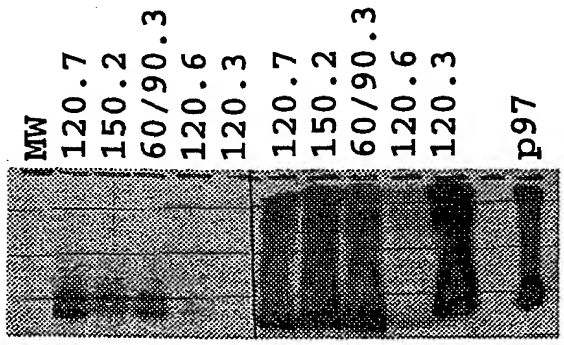


Figure 12c

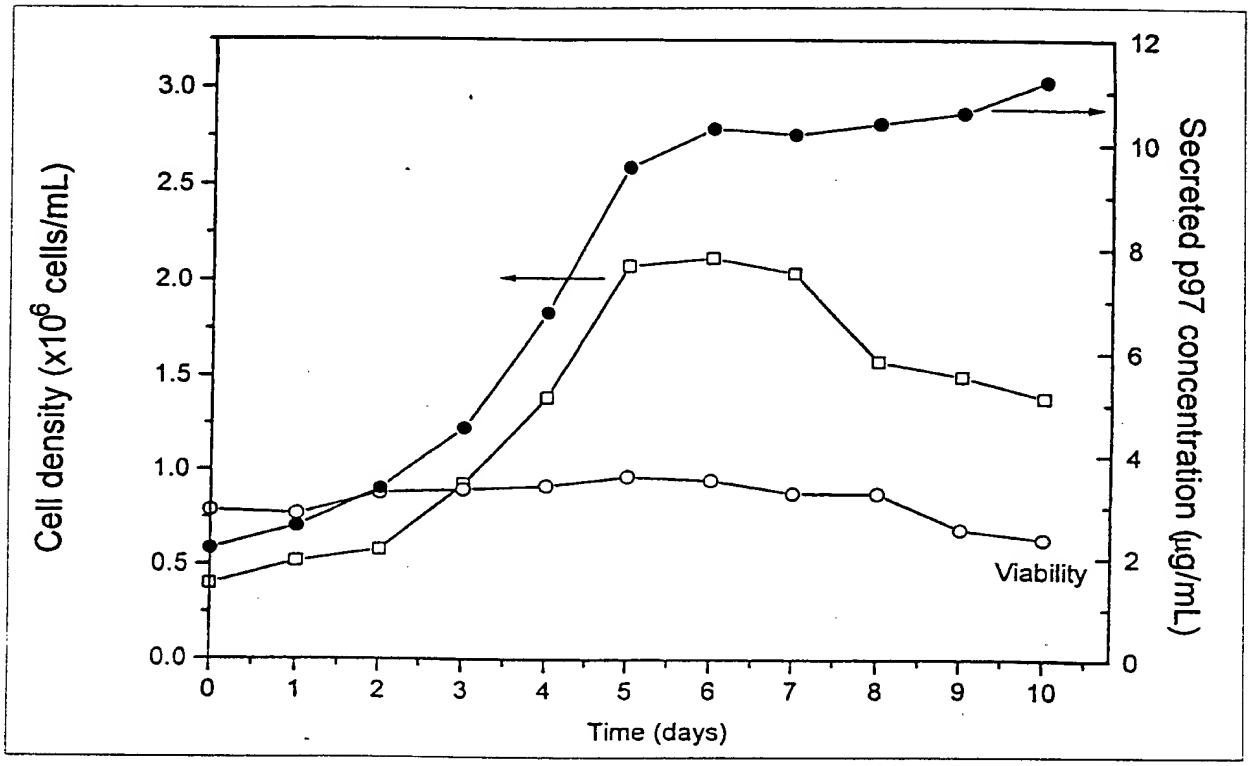

Cell density (x10^6 cells/mL)
Secreted p97 concentration (µg/mL)
Time (days)
Viability
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0
2
4
6
8
10
12
0
1
2
3
4
5
6
7
8
9
10

Figure 12d

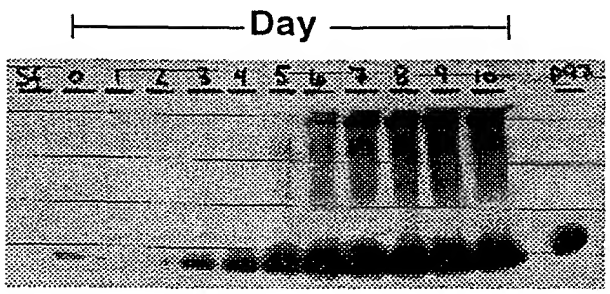


Figure 12e

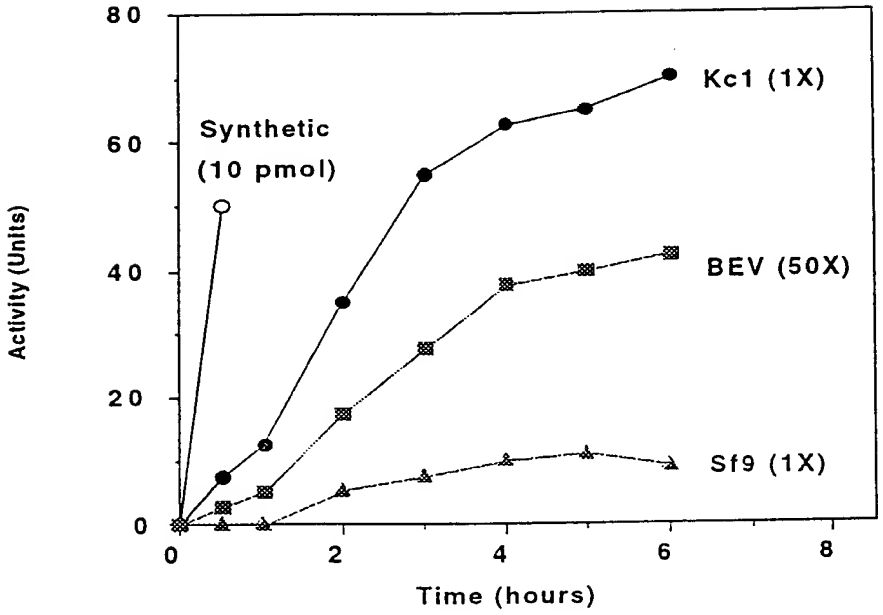

80
60
40
20
0
Activity (Units)
Synthetic
(10 pmol)
Kc1 (1X)
BEV (50X)
Sf9 (1X)
0
2
4
6
8
Time (hours)

Figure 13a

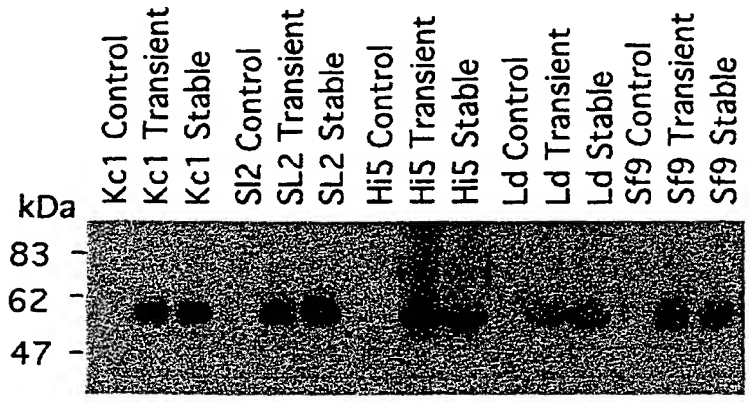

kDa
83
62
47
Kc1 Control
Kc1 Transient
Kc1 Stable
Sl2 Control
SL2 Transient
SL2 Stable
Hi5 Control
Hi5 Transient
Hi5 Stable
Ld Control
Ld Transient
Ld Stable
Sf9 Control
Sf9 Transient
Sf9 Stable

Figure 13b

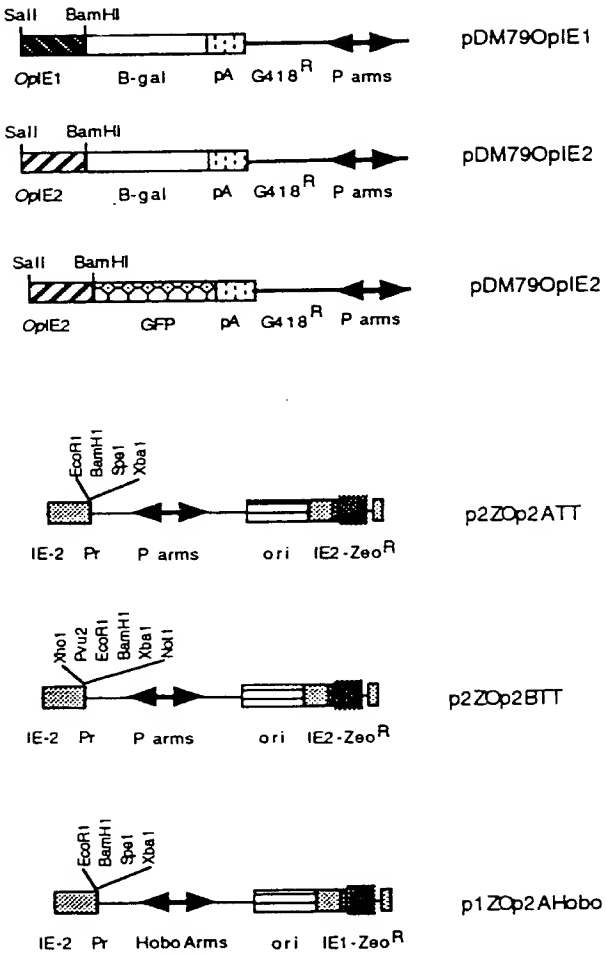

SalI
BamHI
OpIE1
B-gal
pA
G418R
P arms
pDM79OpIE1
SalI
BamHI
OpIE2
B-gal
pA
G418R
P arms
pDM79OpIE2
SalI
BamHI
OpIE2
GFP
pA
G418R
P arms
pDM79OpIE2
EcoR1
BamH1
Spe1
Xba1
IE-2
Pr
P arms
ori
IE2-ZeoR
p2ZOp2ATT
Xho1
Pvu2
EcoR1
BamH1
Xba1
Not1
IE-2
Pr
P arms
ori
IE2-ZeoR
p2ZOp2BTT
EcoR1
BamH1
Spe1
Xba1
IE-2
Pr
Hobo Arms
ori
IE1-ZeoR
p1ZOp2AHobo

Figure 14

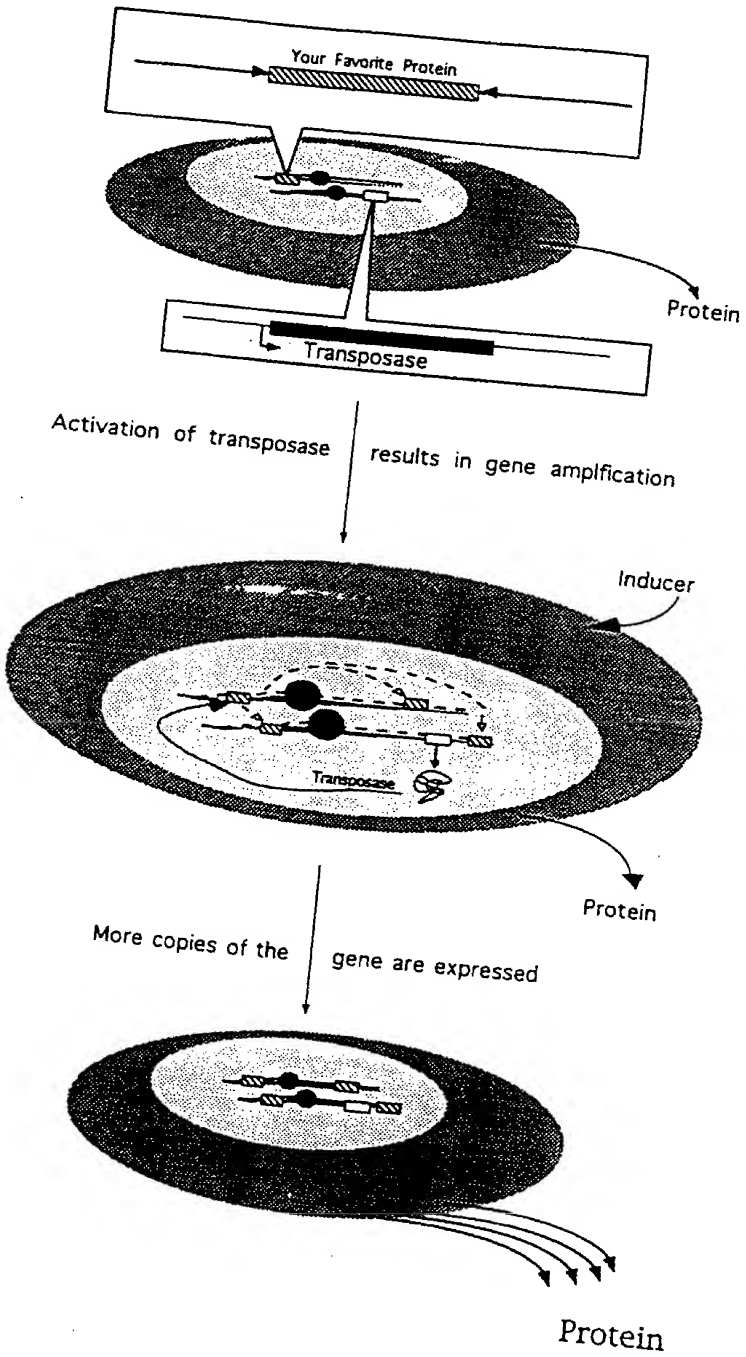

Your Favorite Protein
Protein
Transposase
Activation of transposase
results in gene amplfication
Inducer
Transposase
Protein
More copies of the
gene are expressed
Protein

Figure 15

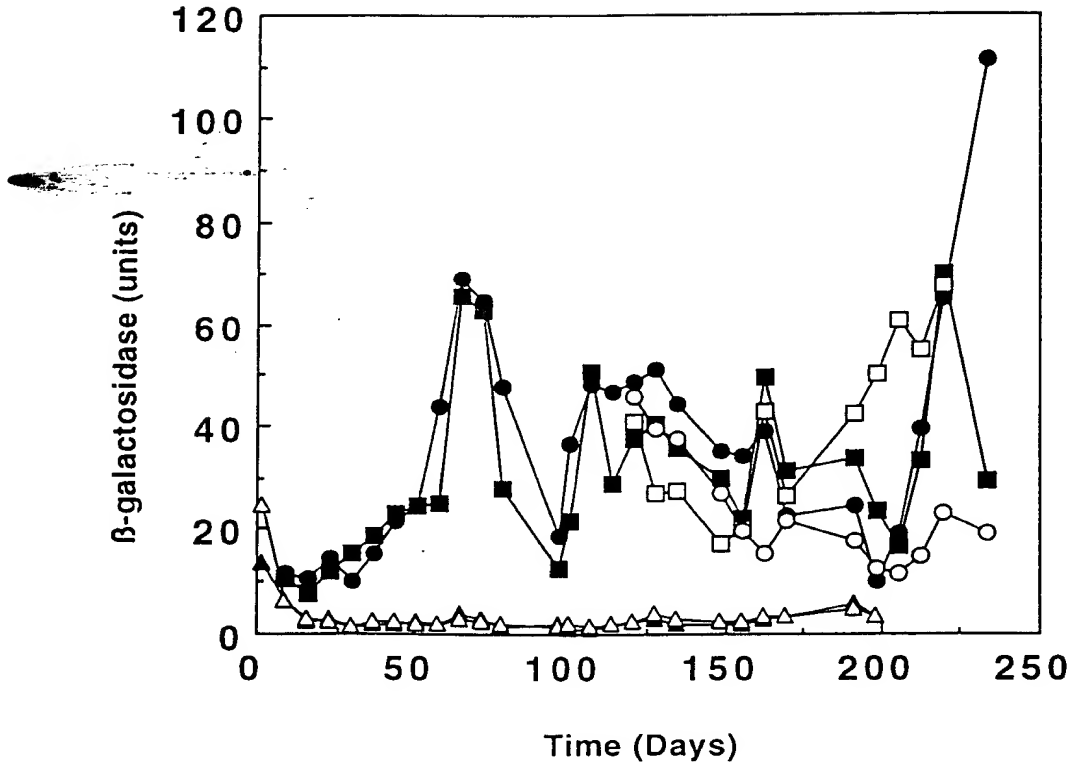

120
100
80
60
40
20
0
ß-galactosidase (units)
0
50
100
150
200
250
Time (Days)

Figure 16a

5' P-element rescue

pDM79
CGACGGGACCACCTTATGTTATTTCATCATGGGCCAGACCCACGTAGTCCAGCGGC...

79-2 Xho.6
CGACGGGACCACCTTATGTTATTTCATCATGTCTCGAACCAACGAGAGCAGTATGC...

79-2 Xho.4
CGACGGGACCACCTTATGTTATTTCATCATGGTACAGACATCTACTTCCCCCCGCT...

79-1 Sph.1
CGACGGGACCACCTTATGTTATTTCATCATGATCTTGCGCTTTAAAATGTGGAGTC...

3' P-element rescue

pDM79
CGACGGGACCACCTTATGTTATTTCATCATGGTCTGGCCATTCTCATCGTGAGCTT...

79-2 kan.3
CGACGGGACCACCTTATGTTATTTCATCATGAGCCAAACAGAAAGCAGAAAAGCTC...

79-2 kan.2
CGACGGGACCACCTTATGTTATTTCATCATGGCCTGACCTAAGCAGATTTGACTGC...

79-2 kan.1
CGACGGGACCACCTT CAACGCTACCTAATCTTAAGAACCA...

consensus

GNCYRRAC

Figure 16b

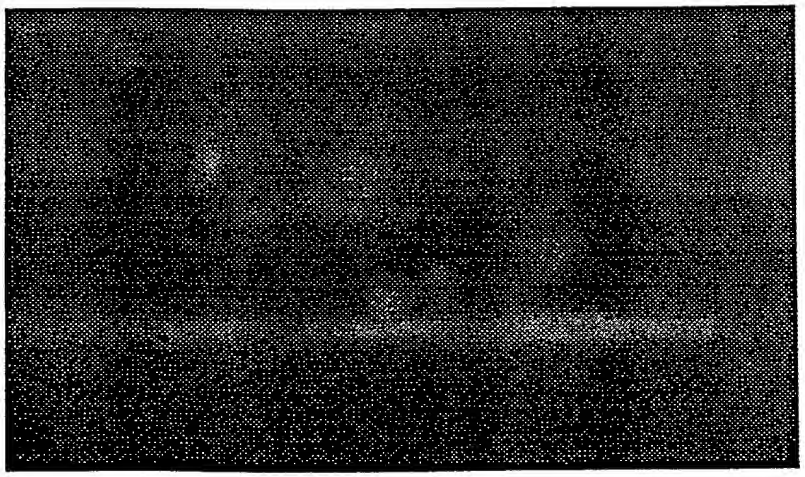


Figure 17